In the Specification

On page 1, please replace the second paragraph with the following:

BACKGROUND

The resolution capability of optical imaging systems is often decisively determined by the object-side aperture of an objective lens and its index of refraction. Light going out from an object can only be detected if it hits the objective within the acceptance angle of the objective. The higher the resolution capability is, the higher the [space] spatial frequencies of the object structure to be imaged which can be detected. The detection of the [space] spatial frequencies is described by the light-optical transfer function or modulation transfer function (in the following: OTF) of the optical systems. The OTF indicates which [space] spatial frequencies, from which the object can be constructed by means of Fourier transformation, are retained in the optical imaging, and/or how parts of the [space] spatial frequencies are attenuated. The resolution capability of the optical system (e.g. a light-optical microscope) is determined by the range in which the OTF of the system does not vanish. If the OTF vanishes completely in sections of reciprocal space, it is impossible, without additional assumptions about the object structure (e.g. spatial limitation, positivity), to reconstruct the corresponding [space] spatial frequencies in an object image. There is general interest in the extension of the OTF in the largest possible region in reciprocal space, in order to increase the resolution of the optical system.

On page 2, please replace the first full paragraph with the following:

In the 4Pi microscope described in EP 0 491 289, there is coherent illumination and, depending on the embodiment, also detection on both sides of the object. In the wave field microscope, typically illumination is performed with coherent plane light waves from opposing sides

(cf. e.g. US-A 4 621 911; F. Lanny et al. in "Bioimaging", Vol. 1, 1993, p. 187 et seq.; US-A 5 801 881). In the I^5M-microscope, there is coherent illumination on both sides and coherent detection, in that the two images of the object are brought into interference on a locally resolving detector (cf. US-A 5 671 085; M. G. L. Gustafsson et al. in "Proceedings of SPIE", Vol. 2655, 1996, p. 62 et seq.). A theta microscope is described by E. H. K. Stelzer et al. in "Opt. Commun.", vol. 111, 1994, p. 536 et seq. and S. Lindeck et al. in "Handbook of Biological Confocal Microscopy", editor J. B. Pawley, Plenum Press, New York 1995, chapter 26, p. 417 et seq., in which light is detected from three sides, with illumination similar to confocal or 4Pi being used. Because the resolution along the [optical] optic axis of the illumination is particularly large for lateral detection in the object plane, one obtains a reduced focused volume overall.

Please replace the paragraph spanning pages 3 and 4 with the following:

Furthermore, many processes (particularly confocal laser scanning, 4Pi, and theta microscopy) are connected with a point by point scanning of the object. This is time-consuming and problematic, above all in the imaging of time-dependent procedures. Scanning processes require very fast detectors (e.g. photomultipliers) which, however, often have a significantly lower detection efficiency than detectors with localized resolution (e.g. CCD's). In fluorescence microscopy, there is the additional problem that the useful illuminance is restricted by the maximum excitation rate of the [pigment] dye in the focus. This additionally restricts the maximum scanning speed.

On page 4, please replace the second full paragraph with the following:

Until now, no significant increase of the resolution capability has been able to be achieved with the processes based on non-linear optical effects. This is particularly associated with the individual photons having to have relatively low energies and therefore large wavelengths to achieve multiphoton absorptions. In addition, the transfer efficiency at higher [space] spatial frequencies is generally very poor, because typically only a very small part of the illumination pattern contains high [space] spatial frequencies.

On page 5, please replace the first full paragraph with the following:

SUMMARY OF THE INVENTION

The basic idea of the invention is, for obtaining an object image (image of an object structure), to record at least two partial images of an object under different object conditions, which are implemented on the object with spatial patterns, with a non-linear function of the light detectable from the object point on the object conditions given at the object point existing for each object point and the partial images containing different contributions of various [space] spatial frequency components of the object structure, and to obtain the desired object image from the partial images through reconstruction of the [space] spatial frequency components. Achieving object conditions with various spatial patterns to detect the various partial images has the advantage that virtual higher and lower frequent [space] spatial frequency components are produced in the pattern of the object conditions to which the [space] spatial frequency components of the object structure are coupled. Due to this coupling, the [space] spatial frequency components of the object structure are displaced relative to the [space] spatial frequency interval which is open for image detection according to the

light-optic transfer function (OTF). The complete object image can be reconstructed from the partial images with a correspondingly expanded [space] spatial frequency range.

On page 6, please replace the first paragraph with the following:

Through the use of a non-linear relationship between the light going out from the object and the local object point related value of a further spatially changing dimension (e.g. the local irradiation or illumination intensity), the [space] spatial frequency range effectively transferred from the system as a whole can be significantly expanded. Through local variation of the influencing variable and the recording of multiple partial images, an object image can be reconstructed whose resolution is, due to the non-linearity, fundamentally higher than the resolution given by the Abbe limit. Various possibilities can be selected according to the application for generating non-linear effects. Obtaining the object image from the partial images is also possible with various types of data evaluation, depending on the application.

On page 7, please replace the first paragraph with the following:

The number of partial images depends on the number of [space] spatial frequency components measurable during the image reconstruction and to be considered, depending on the application, in the pattern of the light sent out from the object. This number is particularly dependent on the object conditions used to introduce the nonlinearity and on the quality of the imaging process realized. If the number of [space] spatial frequency components mentioned is Z, then, as a rule, at least Z partial images are to be recorded. However, depending on the case, it can also be sufficient to record fewer partial images if sufficient information for reconstruction of the object image is present. The number of partial images is permanently set or set automatically depending on the quality of the object image obtained and/or manually by the user of the optical system.

Please replace the paragraph spanning pages 7 and 8 with the following:

The process according to the invention particularly comprises the following steps: (a) adjustment of the conditions obtaining in the object which are able to influence the light going out from an object point in such a way that a non-linear dependence of the light intensity detected from an object point on the value of a spatial pattern contained in at least one object condition is produced in at least one detectable value or a linear dependence to one value at a time of the light intensity detected from this object point on the values of at least two spatial patterns is produced, (b) recording of at least one single image under these object conditions, (c) changing the object conditions in such a way that different [space] spatial frequency components of the object formed by the recording process change in their amplitude and/or phase relationship to one another, (d) recording of at least one further single image under object conditions changed each time according to (c), and (e) evaluation of the measured images, in that the object conditions emphasized differently in the individual images are used to obtain information about the object, associated with [space] spatial frequencies of the object, which was not accessible through simple imaging with the recording process.

On page 8, please replace the third full paragraph with the following:

It is particularly advantageous that high [space] spatial frequencies which are possibly strongly suppressed in imaging objectives can now be detected more efficiently due to the displacement in the frequency space. In addition to the lateral increase in resolution, there is also an axial increase in resolution and the capability of discriminating planes which are perpendicular to the [optical] optic axis in the axial direction. The invention thus provides advantageous usages such as confocal microscopy. With utilization of the non-linear dependency of the quantity of light

detected on the object conditions, the possibility of a significant increase in resolution in the axial direction also results.

On page 9, please replace the second full paragraph with the following:

The invention can be realized using greatly differing non-linear effects. For example, in a fluorescence microscope, illumination can occur with an intensity such that fluorescence [pigments] dyes in the object are saturated. This also allows various [pigments] dyes or [pigments] dyes in various environments (e.g. in various binding states), which can otherwise be differentiated only poorly, to be discriminated on the basis of differing non-linear characteristics (saturation characteristics).

Please replace the paragraph spanning pages 11 and 12 with the following:

A "non-linear dependence" of the light going out from the object and/or detected with a detector is given if its light intensity of the location of the light emission (or scattering or similar activity) does not measurably follow a simple linear model of the current object condition. In a development of functions, e.g. Taylor expansion (see below), of the detected light, terms of higher orders occur. According to the embodiments of the invention mentioned above, a non-linear dependence of the light intensity can be given by one object condition at a time or a linear dependence of the light intensity can be given by multiple object conditions. In the latter case, mixed terms arise in the development of functions which allow the expansion described below of the detectable object [space] spatial frequencies.

On page 12, please replace the third full paragraph with the following:

"Detectable [space] spatial frequency components" are generally understood to mean the components of the frequency space of the Fourier transforms of the objects which are detectable in

principle with the respective imaging processes used.

On page 13, please replace the first paragraph with the following:

Basic principles of image acquisition

Image recording is described in the following with reference to the example of fluorescence microscopy. In fluorescence microscopy, objects which are marked with fluorescence [pigments] dyes or which fluoresce independently are imaged. Depending on the object structure and/or [marking] labeling technique, [marking pigments] labeling dyes collect, for example, in specific sections (e.g. in the cell nucleus of a biological cell). To acquire the image, the object is irradiated with a suitable excitation wavelength and the emitted fluorescence radiation is detected. Fluorescence [pigments] dyes emit with an intensity which is proportional at a first approximation to the intensity of the light irradiated at the location of the [pigments] dyes. In contrast to absorption microscopy, reflection microscopy, or even phase contrast microscopy, the emissions generally occur incoherently to one another. Assuming a proportionality between the fluorescence intensity emitted at an object point to the light intensity of the excitation light irradiated there, a detected image $I_m(\vec{x})$ (converted back into object space coordinates $\vec{x}$) can be described as follows. The position dependent illumination intensity ($Bel(\vec{x})$) is multiplied by the [pigment] dye concentration $Obj(\vec{x})$ presented at the respective object point (object structure) and the result [folded] convolved with the point spread function (PSF) of the imaging system (cf. equation (1))[.]:

$$I_m(\vec{x}) = PSF(\vec{x}) \otimes \left(Bel(\vec{x}) \cdot Obj(\vec{x})\right) \qquad (1).$$

Please replace the paragraph spanning pages 13 and 14 with the following:

In reciprocal space, this translates into the [folding] convolution of the Fourier transformed illumination function $F(Bel(\vec{x}))$ with the Fourier transformed object function $F(Obj((\vec{x}))$ and

subsequent multiplication with the light-optical transfer function $OTF(\vec{k})$ (F refers to the Fourier formation here and in the following, the coordinates in reciprocal space are indicated with $\vec{k}$). Analogously to equation (1), the following results:

$$F(I_m(\vec{x})) = OTF(\vec{k}) \cdot (F9Bel(\vec{x})) \otimes F(Obj(\vec{x}))).$$

On page 14, please replace the second and third full paragraphs with the following:

In typical imaging systems, the range of the OFT not equal to the value zero, which is also referred to as the ["carrier"] "region of support", is restricted by the numeric aperture and the wavelength of the light to be imaged to a specific [space] spatial frequency range (cf. also US-A-5 671 085). Similarly, the Fourier transformation of the illumination function $F(Bel(\vec{x}))$ has the extent of its [carrier] region of support restricted by the light wavelength and, possibly, apertures of the illumination system.

According to the invention, it is thus provided that the effective range of detectable [space] spatial frequencies of the object $F(Obj(\vec{x}))$ ("object [space] spatial frequencies") be expanded on the basis of the following considerations. With the introduction of a non-linear dependence of the light intensity detected on the object conditions, the right part of equation (1) can be written, generalized according to the expressions (2) and/or (3), as follows:

$$PSF(\vec{x}) \otimes I_{em}(Obj(\vec{x}), \vec{b}(\vec{x})) \qquad (2)$$

$$\Leftrightarrow OTF(\vec{k}) \cdot F(I_{em}(Obj(\vec{x}), \vec{b}(\vec{x}))) \qquad (3).$$

Please replace the paragraph spanning pages 15 and 16 with the following:

The Fourier transformation of the illumination intensity $F(Bel(\vec{x}))$ can be represented as the sum of multiple individual δ-functions. Depending on the current illumination pattern, parts of the Fourier transformed object function $F(Obj(\vec{x}))$ are thus displaced by the [folding] convolution with

the Fourier transformed illumination function and added with corresponding weighting. This is illustrated in Fig. 1.

On page 16, please replace the first and second full paragraphs with the following:

Fig. 1 shows the structure of the excitation distribution in reciprocal space with sinusoidally distributed, low illumination intensity corresponding to a typical spatially patterned illumination. The arrows pointed upward indicate the maxima which result from the sinusoidal excitation (Fourier transformation of the sine function). In addition, the structure of the Fourier transformations of the object function $F(Obj_{+1}(k))$ "coupled" to the maximum k_p is indicated. The Fourier transformations of the object function coupled to the other maxima are not indicated for reasons of clarity. Actually, however, $Obj(\vec{k})$ is "coupled" to each virtual maximum because $Obj(\vec{k})$ is to be [folded] convolved with the virtual [space] spatial frequency components in the pattern of the object conditions in reciprocal space. Only the central portion of emitted [space] spatial frequencies (indicated as the [carrier] region of support of the OTF) is accessible to detection.

How the detectable range is established ("punched out") from the sum corresponding to the [folding] convolution mentioned by the optical imaging (multiplication with the [space] spatial frequency limited OTF) is illustrated as an example in Fig. 1. The range of detectable object [space] spatial frequencies is significantly expanded for illumination with a specific pattern relative to the case of a uniform illumination. With the reconstruction process described below, the displaced object [space] spatial frequencies can again be combined into a consistent image.

Please replace the paragraph spanning pages 16 and 17 with the following:

According to equation (4), in the non-linear case, terms of higher order in $b_1(\vec{x})$ also provide contributions to I_{em}, such as the terms with the factors c_5 and/or c_6. The Fourier transformations of

these terms are also contained in $F(I_{em}(Obj(\vec{x}), \vec{b}(\vec{x})))$. With $b_1(\vec{x}) = Bel(\vec{x})$, one also obtains the term $c_5 \cdot [[](F(Bel(\vec{x})) \otimes F(Bel(\vec{x}))[]]) \otimes F(Obj(\vec{x})$ in the expression 2. With a certain component in the image, it is now possible to measure [space] spatial frequencies of the object which were previously not accessible, because they could not yet be displaced, by [folding] convolution with the [space] spatial frequency limited function $F(Bel(\vec{x}))$, into the range detectable by means of OTF. The extent of the [carrier] region of support from $F(Bel(\vec{x})) \otimes F(Bel(\vec{x}))$ can now, however, be correspondingly larger, with higher [space] spatial frequencies thereby also displacing into the range corresponding to the OFT and thus being measurable in the image. Further higher orders work out correspondingly in further [foldings] convolutions with the Fourier transformations of $b_i(\vec{x})$, so that even higher object [space] spatial frequencies are detectable. In principle, it is possible to detect [space] spatial frequencies of the object of any desired height and thereby to increase the resolution as much as desired, if corresponding coefficients are present in the series expansion according to equation (4). However, in practice, the resolution achievable during reconstruction is often restricted by the signal-noise ratio attainable at the high object [space] spatial frequencies.

Please replace the paragraph spanning pages 17 and 18 with the following:

The effective occurrence of higher and lower frequency components of the illumination pattern in reciprocal space is illustrated in Fig. 2 with reference to the example of fluorescence microscopy. If the object is irradiated with a sufficiently high illumination intensity, a non-linear dependence of the fluorescence emission on the excitation intensity (saturation of the fluorescence) results and therefore a pattern of excitability of fluorescence (in the following: excitation pattern) of a specific [pigment] dye, which consists in principle of infinitely many (virtual) maxima in reciprocal space, whose absolute height quickly falls, however, as $|\vec{k}|$ increases. As explained above

in the example of the relatively weak excitation intensity (linear case, Fig. 1), the object function is coupled to each of the components of the illumination function. All information, particularly about the high-frequency local frequencies of interest of the object function, is therefore contained in a detector signal recorded as partial image I_{em}. The reclamation of this information is described below in connection with image reconstruction.

On page 18, please replace the first full paragraph with the following:

Fig. 2 illustrates the structure of the excitation distribution in reciprocal space for a non-linearly distorted excitation pattern. A coupled structure of the Fourier transformations of the object function ($Obj_{+2}(\vec{k})$) is drawn to the virtual maximum n = +2 corresponding to $2k_0$. For practical purposes, $Obj(\vec{k})$ is in turn coupled to each maximum (not shown for reasons of clarity) and overlapped with displaced, varyingly intensive, and phase-shifted versions of itself. Only the central portion of emitted [space] spatial frequencies ([carrier] region of support of the OTF) is accessible to detection.

On page 19, please replace the second and third full paragraphs with the following:

Reconstruction of the object image

According to a preferred embodiment of the invention, the terms contained in the Taylor expansion according to equation (4) are established by the solution of an equation system and thus separated from one another, if they have a measurable influence. The equation system, whose determination is explained in detail below, can be determined and, in principle, solved at each point in the range of the [carrier] region of support of the OTF in reciprocal space, in spite of multiplication with the OTF.

Through displacement in Fourier space (or through multiplication with $\exp(i\vec{\Delta k}\vec{x})$ in the [local] real space, $\vec{\Delta k}$: frequency space displacement vector), the individual components can then be combined in such a way that a high-resolution image results. This can, if necessary, be processed with further deconvolution techniques in order to further increase the image quality.

Please replace the paragraph spanning pages 19 and 20 with the following:

As already known from US-A-5 671 085, the illumination of the object with a pattern made of the highest possible [space] spatial frequencies results in an increase in resolution relative to typical light-optical microscopy. Through the utilization according to the invention of a non-linear relationship between the strength of the pattern, e.g. values of specific object conditions, at an object point and the light intensity going out (emitted and/or diffracted) from this object point, it is possible to calculate an image with an even higher [local] spatial resolution.

On page 20, please replace the first full paragraph with the following:

An example for the formation of a pattern of object conditions is given in the excitation of fluorescence with a position-dependent distribution of intensive excitation light. The non-linear dependence of the light detected by the detector can, for example, occur due to the saturation of the excitation of fluorescence [pigments] dyes present in the object. If the excitation light has a sufficiently high intensity, one obtains a non-linear relationship between the irradiated and the emitted light intensity on the object observed (cf., for example, D. R. Sandison et al. in "Handbook of Biological Confocal Microscopy", Plenum Press, New York and London, 2nd edition, 1995, chapter 3, pp. 47 to 50: and R. Y. Tsien et al., in the "Handbook of Biologicl Confocal Microscopy" cited, chapter 16). The detected light thus also contains information about [space] spatial frequencies of the object which would otherwise not be accessible. However, each image recorded

in this way contains a mixture of components of higher [space] spatial frequencies, which can then, however, be separated and combined into a consistent, high-resolution image by recording under varying conditions and counterbalancing multiple partial images.

Please replace the paragraph spanning pages 20 and 21 with the following:

The intensity distribution of the excitation light is approximately described in this example by a sine function displaced into the positive range. In the ideal case, point-shaped maxima at $\vec{k}=0$, $\vec{k}=+\vec{k}_b$, and $\vec{k}=+\vec{k}_b$ result as Fourier transformations (cf. Fig. 1). These maxima have, depending on the degree of modulation, a specific energy and a specific phase angle in the complex plane, which depends on the position and/or the displacement (location) of the pattern of the excitation light. Through the Influence of the non-linear dependence of the fluorescence emissions on the excitation intensity (saturation of the fluorescence), the Pattern shown in Fig. 2 with lower and higher frequency components in reciprocal space results, for example, as the excitability pattern for a specific [fluorophor] fluorophore types.

On page 21, please replace the first full paragraph with the following:

For reconstruction of the object image, it is sufficient to truncate approximately at a finite [space] spatial frequency value $\vec{k}_{max} = \pm m\vec{k}_b$ and to consider only maima with smaller [space] spatial frequencies in the calculation.

Please replace the paragraph spanning pages 21 and 22 with the following:

If the excitation light pattern is displaced relative to the object, the respective complex [phase locations] phases of the individual point-shaped maxima in the Fourier space change. If one considers ± m excitation maxima and the maximum at $\vec{k} = 0$, one therefore requires $Z = 2m + 1$ images recorded under various conditions in order to be able to separate the individual components

of the object image through the microscope, which are [folded] convolved (i.e. displaced) with the current maximum (in Figs. 2, only one component is illustrated). It can, for example, be provided that Z = 5 maxima are considered. The phase angle of the maxima in the frequency space of the excitation pattern moves upon displacement of the pattern proportionally to $n|\vec{k}_b|$, because a displacement in the space by $\vec{\Delta x}$ corresponds to a multiplication in the frequency space with $\exp(i\vec{k}\vec{\Delta x})$. In this case, n corresponds to the number of the current [space] spatial frequency component (cf. Fig. 2). Therefore, if various images (partial image) $I_n(\vec{k}) = F(I_n(\vec{x}))$ of the object are recorded, each with the phase [location] of the illumination pattern (i.e. the excitation pattern) displaced by a fifth of the basic pattern relative to one another, the following equation system results:

$$M \cdot \begin{pmatrix} Obj_0(\vec{k}) \\ Obj_{+1}(\vec{k}) \\ Obj_{-1}(\vec{k}) \\ Obj_{+2}(\vec{k}) \\ Obj_{-2}(\vec{k}) \end{pmatrix} = const \cdot \begin{pmatrix} I_0(\vec{k}) \\ I_1(\vec{k}) \\ I_2(\vec{k}) \\ I_3(\vec{k}) \\ I_4(\vec{k}) \end{pmatrix}$$

$$M = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ 1 & \exp(i2\pi / 5) & \exp(-i2\pi / 5) & \exp(i4\pi / 5) & \exp(-i4\pi / 5) \\ 1 & \exp(i4\pi / 5) & \exp(-i4\pi / 5) & \exp(i8\pi / 5) & \exp(-i8\pi / 5) \\ 1 & \exp(i6\pi / 5) & \exp(-i6\pi / 5) & \exp(i12\pi / 5) & \exp(-i12\pi / 5) \\ 1 & \exp(i8\pi / 5) & \exp(-i8\pi / 5) & \exp(i16\pi / 5) & \exp(-i16\pi / 5) \end{bmatrix}$$

On page 22, please replace the first full paragraph with the following:

In this equation system, $Obj_n(\vec{k})$ corresponds to the displaced complex value components of the Fourier transformed object (object [space] spatial frequencies) belonging to the n-th maximum of the excitation pattern, which are then transmitted by the OTF of the imaging system.

On page 23, please replace the second through fourth full paragraphs with the following:

The complex value components $Obj_n(\vec{k})$ can now be displaced by the vector $\vec{\Delta k}$ in the Fourier space (or by the corresponding multiplication in real space discussed above), so that the respective [space] spatial frequency $\vec{k}$ ends up where it would be measured in a non-patterned, uniform illumination. $\vec{\Delta k}$ is therefore $-n\vec{k}_b$ in this case.

In a further step, a correction of the components $Obj_n(\vec{k})$ in their complex [phase locations] phases is provided, according to the mutual phase [location] ϕ_n of the frequency space excitation maxima in the image I_n (multiplication with $\exp(-i\phi_n)$). Subsequently, the components $Obj_n(\vec{k})$ are combined, if necessary through weighted addition, into a consistent image (the desired object image). In this way, an extension of the [carrier] region of support of the entire OTF to a range significantly enlarged relative to the linear image and therefore an increase of the resolution capability is made possible.

The displacement of [space] spatial frequency components illustrated can be performed in different space directions. This can occur successively through varying orientation of the illumination pattern or simultaneously through illumination with a multidimensional structure. The resolution capability can be increased in one, two, or three dimensions. The overall transfer function can be altered still further, subsequently or in intermediate steps, by appropriate filters and/or application of unfolding techniques known per se.

On page 26, please replace the first full paragraph with the following:

The illumination optic 30 has an excitation filter 31, a [dichroitic] dichroic mirror 32 for coupling the excitation light into a microscope column and objective lenses 33. At the location of the object 40, an image of the diffraction grating 22 is formed in the corresponding focal plane as

illumination for the sample to be examined. The imaging lens 50 is formed in turn by the objective lenses 33, an emission filter 51, and an optionally provided optic 52 for image enlargement. The detector device 60 is a CCD detector, by which data is transmitted to the image generator (not shown).

Please replace the paragraph spanning pages 26 and 27 with the following:

For the recording according to the invention of multiple partial images with different [phase locations] phases, the diffraction grating 22 is displaced in small steps relative to the object 40. The step width depends on the structure dimensions of the mask and the number of partial images to be recorded and is, for example, 30/7 μm for a 30 μm structure dimension and 7 partial images. Alternatively, a displacement of the object 40 can be provided for a fixed diffraction grating 22, with this, however, requiring additional steps of image correction during the reconstruction of the object image. Alternatively, it is also possible to influence the phase of the various diffraction maxima directly through suitable optical elements. The minimum number of exposures of partial images necessary for the reconstruction of the complete image results from the number of unknowns of the associated equation system (see above). At least two partial image exposures are intended.

On page 27, please replace the first through third full paragraphs with the following:

In order to increase the resolution in all spatial directions, the object 40 is illuminated successively with patterns at various angles or with a mask, such as with the DMD or LCD device with a two-dimensional pattern which produces the diffraction maxima in multiple directions of the plane, in various [phase locations] phases in each dimension.

By providing a series of focuses, one can obtain still more information about the axial structure of the object and thereby derive three-dimensional object images. This is simplified even

more, on one hand, by the incoherent light source and, on the other hand, by the presence of the zero [point] diffraction order of the grating. A further increase in resolution can be achieved by rotating the object under the optical system around an axis perpendicular to the [optical] optic axis.

The object 40 is illuminated and/or excited with instantaneous intensities in such a way that the pigments in the sample become saturated, so that the desired non-linear effect for increasing the resolution capability results. The components of the overlapping individual orders sought can be calculated from the images for various [phase locations] phases of the excitation structure. It is also possible to reconstruct high-resolution images from exposures of partial images with varying illumination intensity. If one suppresses the zero [point] diffraction order of the diffraction grating 21 (e.g. by masking), one thereby advantageously increases the degree of modulation of the illumination function and therefore the relative intensity in higher orders of excitation. In addition, the energy can be displaced into higher [space] spatial frequency ranges.

On page 28, please replace the second paragraph with the following:

To change the object conditions (interference pattern at the object 40) to record various partial images, at least two mirrors are positioned movably. For example, it is provided that the mirrors 24 and 25 are displaceable for changing of the interference pattern. Alternatively, at least one electrooptical element for changing the phase [location] of the illumination light is provided in one of the partial light paths for changing the object conditions.

Please replace the paragraph spanning pages 29 and 30 with the following:

The exemplary embodiments described are based on the usage of the non-linear dependence of the light detected on the intensity of the excitation light due to saturation of fluorescence pigments. Alternative non-linear effects are given by the saturation of the absorption of excitation

light under intensive illumination, the dependence of the phase of the emitted or scattered light on the illumination intensity present in the object, which converts in the detector (e.g. the interference) or before it into a non-linear intensity dependence, SHG or THG processes, a dependence of the light characteristics of the Raman scattering on the value of one or more object conditions, temporally coherent effects (e.g. Rabi oscillations) on atoms or molecules in the object, CARS processes, multiphoton absorptions, stimulated emissions in the object, the population of longer-lived excitation states or chemically altered states in the [fluorophors] fluorophores before or during the illumination, radiation-free energy transfer processes, and/or physical or chemical object conditions.

On page 30, please replace the first full paragraph with the following:

Particularly for the use of temporally coherent effects (Rabi oscillations) on atoms or molecules or [fluorophors] fluorophores in the object (in solution, in solid bodies, in gases, or under vacuum conditions), illumination devices with extremely short pulse lengths (e.g. < 100 fs) are preferably used. If the non-linear effect is based on stimulated emission, this is induced simultaneously or in temporal sequence. The stimulated emission can be induced at the same wavelength as that of the excitation light or at other wavelengths, e.g. at a typical fluorescence wavelength. The usage of energy transfer processes means that energy of the excitation radiation is transmitted with or without radiation by [fluorophors] fluorophores onto neighboring [fluorophor] fluorophore molecules and thereby a multilinear dependence of the emitted light intensity on the intensity irradiated onto the neighboring location arises.

Please replace the paragraph spanning pages 31 and 32 with the following:

Figs. 6 and 7 illustrate simulation results for the use of a fluorescence microscope according to Fig. 4. The light intensity is represented here as blackening. For reasons of print technology, the

image quality is restricted. For better visualization of the illumination, a constant background fluorescence is assumed in the object (Figs. 6a, 7a). Fig. 6a shows the simulated object, whose illumination with the processes described and imaging with an epifluorescence microscope is simulated. The partial images b-h represents simulations of partial images, each taken with different phases of the illuminating line pattern. Fig. 6i shows an example in which the direction of the illuminating pattern is also changed. The excitation intensity was larger by a factor of 5 than the saturation intensity in this simulation. The maximum expected photon count was 560 photons/pixel in the individual images. Fig. 7 shows the associated reconstruction results. Fig. 7a repeats the original image of the simulation. After filtering with the point spread function of a simulated microscope and a Poisson distributed increase of noise (maximum = 560 photons), the image from Fig. 7b results. The reconstruction according to the invention from partial images, which was simulated with illumination patterns under 3 rotational angles analogously to the Figs. 6b to 6h, is shown in Fig. 7c. In this case, no amplification of higher [space] spatial frequencies is performed. If the high frequency amplification intrinsic to the system also occurs, the image according to Fig. 7e results from Fig. 7c. The usage of a corresponding high-frequency amplification with the (convention) image according to Fig. 7b would merely result in an image according to Fig. 7d. The comparison of the images of Fig. 7e and Fig. 7d shows the superiority of the process according to the invention of reconstruction of higher [space] spatial frequencies from the recording of multiple partial images. Fig. 7f illustrates the associated simulated carriers of the OTF achieved with the process for a number of maxima Z = 7 considered.

Please replace the paragraph spanning pages 32 and 33 with the following:

Fig. 9 shows a lateral section through the simulated effective optical transfer function of the

overall system according to Fig. 4. The grating interval of the diffraction grating is selected here in such a way that only the diffraction orders 0, +1, and –1 of the diffraction grating can be transmitted by the objective. Through the partial saturation of the pigments involved, a non-linear relationship between the excitation intensity and the probability of excitation of a pigment molecule at one point in the object space results. This spatially varying excitation probability is also referred to as the excitation pattern. If one assumes that the excitation probability for a specific pigment molecule is a function of the excitation intensity, then a nonlinearity of this function leads to spatially higher harmonics of the excitation pattern also occurring in the emission pattern. Maxima in reciprocal space which lie beyond the [space] spatial frequency limit given by the Abbe limit can then occur in the excitation pattern. The [space] spatial frequency limited imaging of the multiplication of the pigment distribution with the excitation pattern now contains components analogous to a linear excitation with a pattern containing higher [space] spatial frequencies.

On page 33, please replace the second full paragraph with the following:

Fig. 10 shows a simulated application of the process according to the invention on a sectional image of the cell nucleus of an embryonic bovine cell recorded by means of electron microscopy. Fig. 10a illustrates the inverted electron microscope section near the nuclear membrane with the nuclear matrix. The simulated epifluorescence microscopy exposure with [unfolding] deconvolution results in the typical image shown in Fig. 10b. With use of the process according to the invention, the image shown in Fig. 10c results. The image taken and evaluated with the method of saturated lateral modulation and subsequently [unfolded] deconvolved (maximum: 560 photons in the single image) is significantly improved compared to the typical image and comparable with the original electron microscope image.